



Design of Fab-based chimeric antibodies against Bothrops asper toxins

M. Haack, Aleksander; B. Hallgren, Malte; U. W. Friis, Rasmus; H. Dam, Søren; Martos Esteban, Andrea; Andersen, Mikael Rørdam; Kilstrup, Mogens; Laustsen, Andreas Hougaard

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
M. Haack, A., B. Hallgren, M., U. W. Friis, R., H. Dam, S., Martos Esteban, A., Andersen, M. R., Kilstrup, M., & Laustsen, A. H. (2016). *Design of Fab-based chimeric antibodies against Bothrops asper toxins*. Poster session presented at Symposium for Biological and Life Science Students 2016, Cambridge, United Kingdom.

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Design of Fab-based chimeric antibodies against *Bothrops asper* toxins

Aleksander M. Haack^{1,2}, Malte B. Hallgren^{1,2}, Rasmus U. W. Friis^{1,2}, Søren H. Dam^{1,2}, Andrea Martos Esteban¹, Mikael Rørdam Andersen¹, Mogen Kilstrup¹, Andreas H. Laustsen¹

¹Department of Biotechnology and Biomedicine, Technical University of Denmark

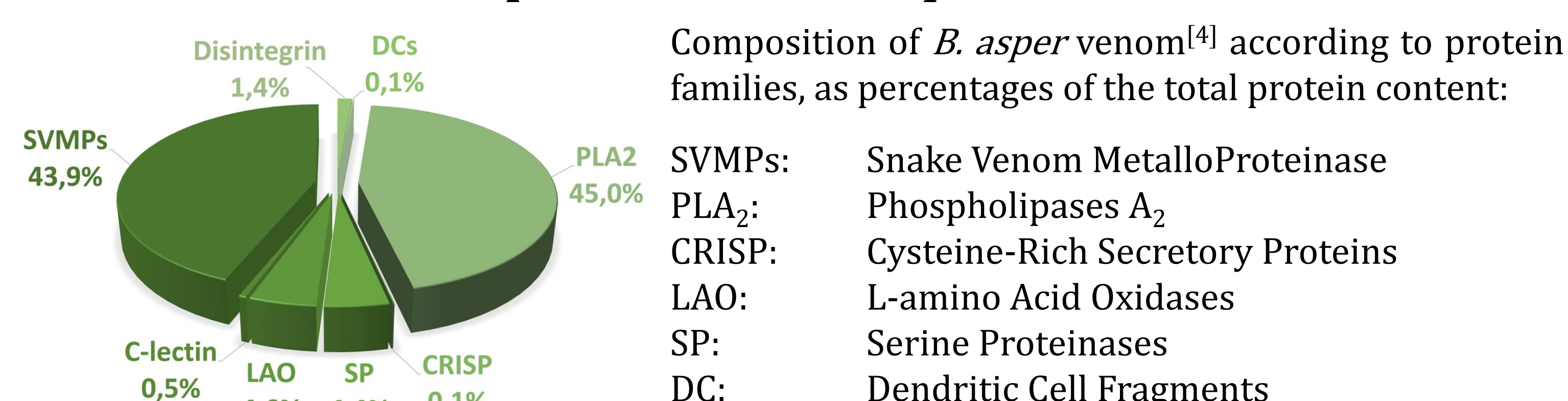
²Department of Bio and Health Informatics, Technical University of Denmark

1 Addressing the problem of immunogenic antivenoms

Snakebite is one of the world's most neglected tropical diseases, with an estimated 5 million bites per year, resulting in about 125.000 deaths.^[1] The only current treatment for snakebite envenoming is antiserum derived from the blood of immunized mammals (typically horses).^[2] These antisera are expensive to produce and carry a high risk of causing hyper-allergic reactions in human recipients due to their heterologous origin.^[3] Here we report the discovery of chimeric scFvs against *Bothrops asper* toxins.



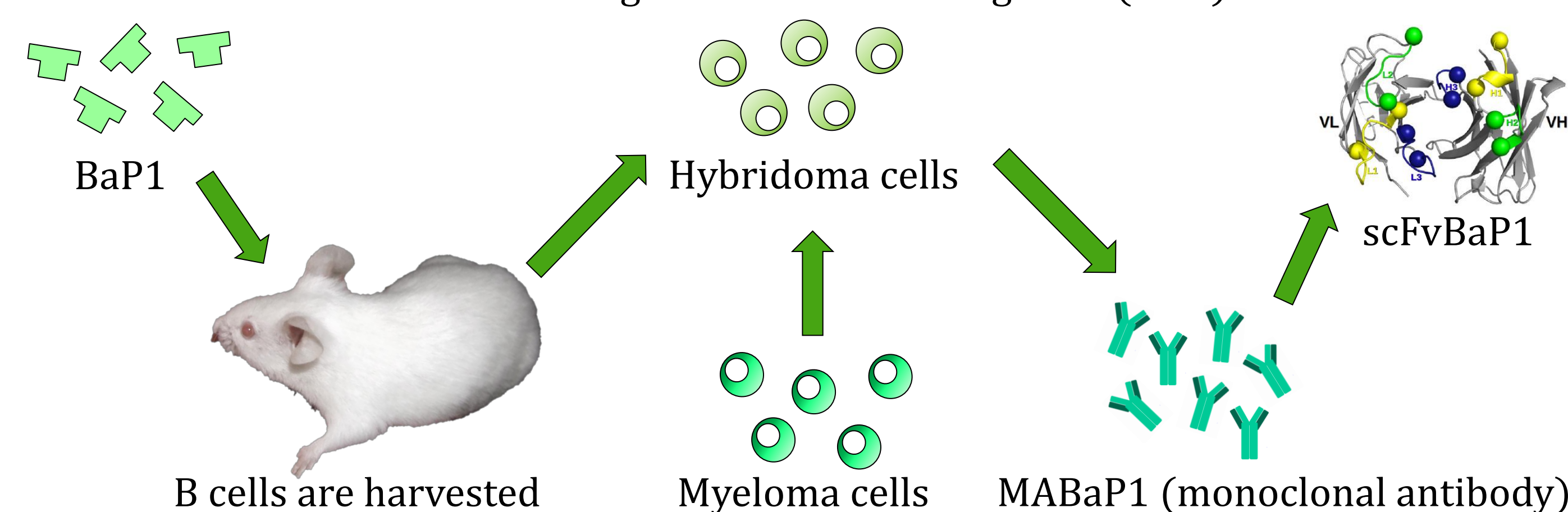
2 Composition of *B. asper* venom



Results presented here were obtained prior to the project^[4]

3 Construction of scFvBaP1 from a monoclonal antibody

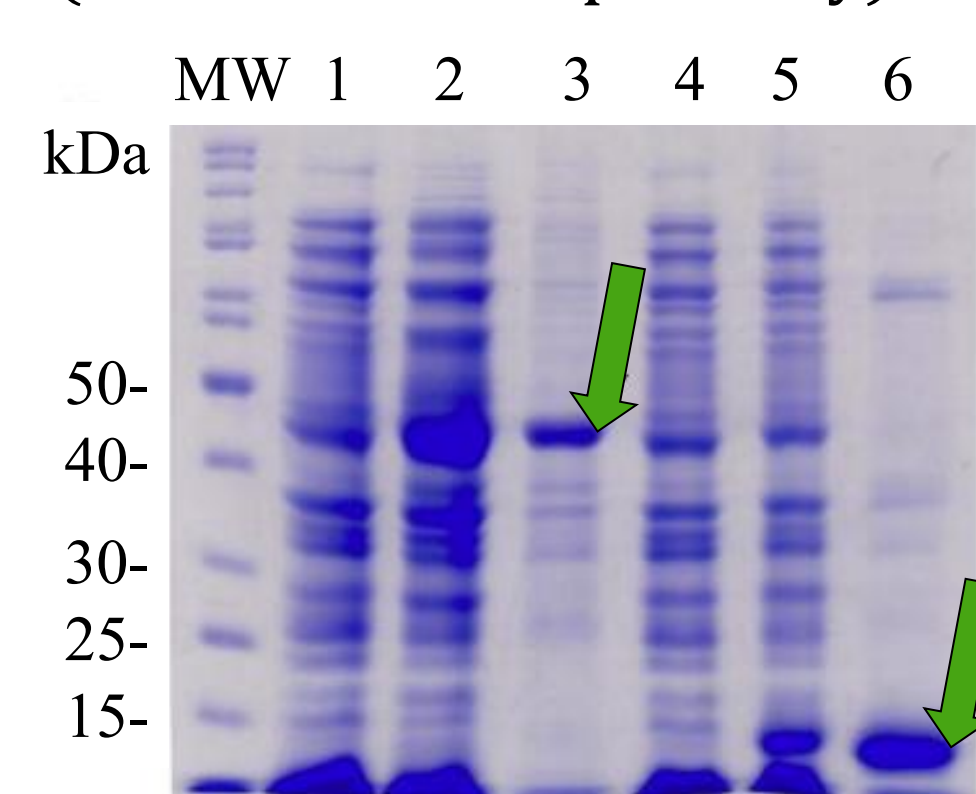
Hybridoma cells secreting the monoclonal antibody against BaP1 (MABaP1) were made, and from these a recombinant single chain variable fragment (scFv) was constructed.



Results presented here were obtained prior to the project^[5]

4 Results from the recombinant scFvBaP1

The scFvBaP1 was cloned into the pMST3 expression vector in frame with a SUMO (Small Ubiquitin-like Modifier) sequence. When subjected to SDS-PAGE, the sequenced SUMO-scFvBaP1 showed a band at 40 kDa as was predicted and SUMO protein around 13 kDa^[5] (well 3 and 6 respectively).

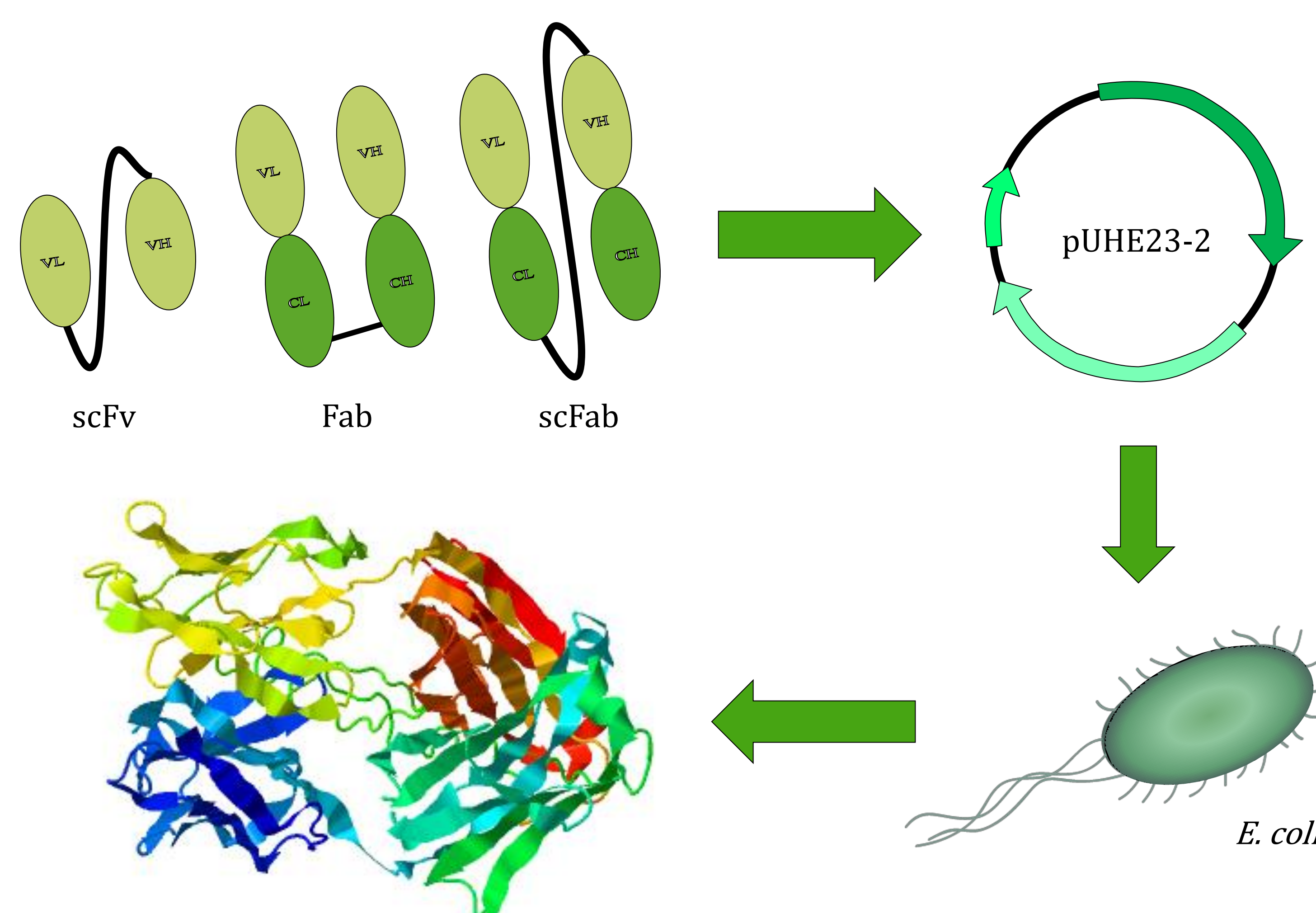


In vivo tests showed that scFvBaP1 specifically recognizes BaP1 and whole *B. asper* venom, and to a large extent neutralizes the main toxic effects of BaP1.

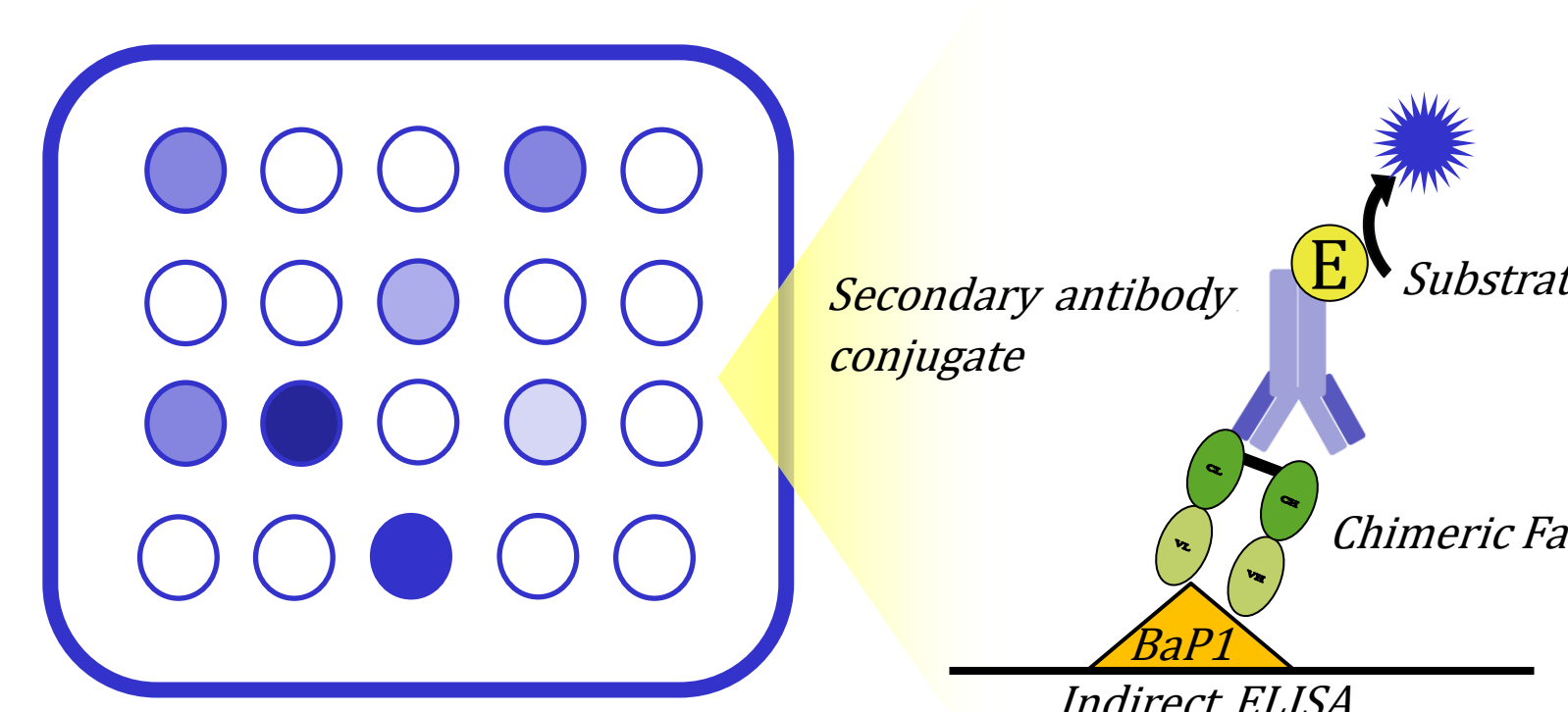
Results presented here were obtained prior to the project^[5]

5 Transformation of antibody formats into *E. coli*

Recombinant FabBaP1 and scFabBaP1 will be made from scFvBaP1. These are going to be expressed in *Escherichia coli* using a pUHE23-2 expression vector. The final protein model shown here is of the recombinant scFab.^[6]



6 Binding strength testing of chimeric antibodies by ELISA



Binding strengths of the antibodies to BaP1 will be determined by ELISA. This will allow us to compare the different antibody formats in regards to how likely they are to neutralize BaP1.

7 Next steps: Determining the best antibody format

If good chimeric antibodies are obtained in good yield, the next steps will be to determine their K_D and test the antibodies in a preclinical model. This will allow an *in vivo* comparison between the different formats.

References

- [1] Richard G., Meyers A.J., McLean M.D., Arbabi-Gahroudi M., MacKenzie R., et al. (2013) In Vivo Neutralization of α -Cobratoxin with High-Affinity Llama Single-Domain Antibodies (V_HHs) and a V_HH-Fc Antibody. PLoS ONE 8.7:e69495
- [2] Gutiérrez, I.M., León, G., Lomonte, B., Angulo, Y. (2011) Antivenoms for snakebite envenomings. Inflammation and Allergy - Drug Targets 10.5:369-380
- [3] Harrison R.A., Cook, D.A., Renjifo, C., Casewell N.R., Currier R.B., Wagstaff, S.C. (2011) Research strategies to improve snakebite treatment: Challenges and progress. Journal of Proteomics 74.9:1768-1780
- [4] Alape-Giron A., Sanz L., Escolano J., Flores-Díaz M., Madrigal M., Sasa M., Calvete J.J., et al. (2008) Snake Venomics of the Lancehead Pitviper Bothrops Asper: Geographic, Individual, and Ontogenetic Variations. Journal of Proteome Research 7.8:3556-3571
- [5] Castro J.M.A., Oliveira T.S., Silveira C.R.F., Caporino M.C., Rodriguez D. et al. (2014) A neutralizing recombinant single chain antibody, scFv, against BaP1, A P-I hemorrhagic metalloproteinase from Bothrops asper snake venom. Toxicon 87:81-91
- [6] I-TASSER, protein modeling tool, <http://zhanglab.cmb.med.umich.edu/I-TASSER/> [Accessed 9th of November]

Contact information

Aleksander M. Haack: s153292@student.dtu.dk
Søren H. Dam: s153398@student.dtu.dk
Malte Hallgren: s153002@student.dtu.dk
Rasmus U.W. Friis: s154269@student.dtu.dk

Acknowledgements

We thank the Novo Nordisk Foundation (NNF160C0019248) and Symphogen A/S for financial support.